Vanderbilt Pre-cancer Atlas HTAN Center: Biology Underlying the Initiation of Colorectal Cancer within a Diverse Population

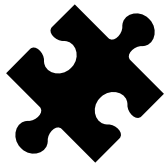
Martha J. Shrubsole, PhD
on behalf of the Vanderbilt Pre-Cancer Atlas
Vanderbilt University Medical Center
Vanderbilt University
Nashville, TN


Bob Coffey


Ken Lau


Martha Shrubsole

Challenges and Strategies for Colorectal Cancer



- Needs
  - Risk stratification – who is at high risk?
  - Improved screening modalities
  - Early detection strategies
  - Prevention or interception strategies including chemoprevention

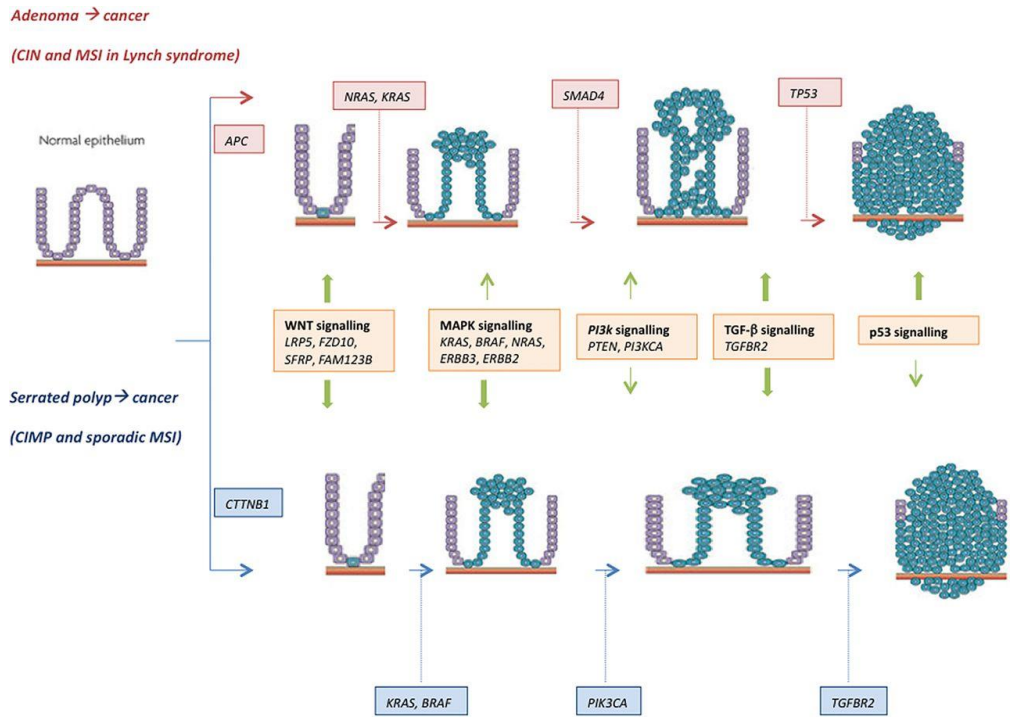
- Potential Solutions
  - Understanding of molecular phenotypes
  - Understanding of progression of adenomas

Pathways to Colorectal Cancer

Precursor Lesion

Conventional Adenoma

Sessile Serrated Polyp



65-80% of CRCs
- CIN; MSS

10-35% of CRCs
- CIMP; MSI-H

Dickinson et al. Gut 2015;64:1485-1494
Guinney et al. Nature Medicine 2015 Nov;21(11):1350-6

COLON MAP – *The Colorectal Molecular Atlas Project*

VANDERBILT UNIVERSITY MEDICAL CENTER

An integrative single cell atlas of host and microenvironment in colorectal neoplastic transformation

An NCI Cancer Moonshot℠ Project

Study Design

- 3D modeling for progression of sporadic colon adenoma to colon cancer
  - scRNA-seq for identifying cell state markers
  - Spatial profiling/analysis using MxIF and smFISH
  - Multiregional exome sequencing
  - Biofilm analysis
- Enrollment of participants from colonoscopy and surgery

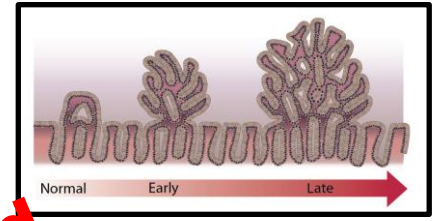
Biospecimens

- Colon tissue:
  - Polyps and normal mucosa
  - Fresh, FFPE, Carnoy's fixed (for microbial biofilm analysis)
  - TMAs, whole sections
- Blood, urine, stool, oral swab, oral rinse

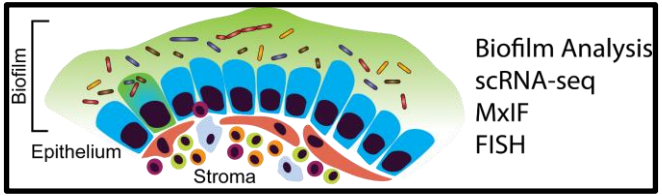
Participant-Level Data

- Standardized pathology
- Diet and lifestyle factors
- Clinical history and findings

Conventional Adenoma and Sessile Serrated Polyps

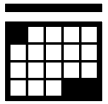


Goal: Modeling progression of sporadic colon polyp to colon cancer with spatial information on the microbiome and host cells



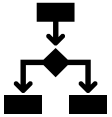
PI UNIT	BIOSPECIMEN UNIT	CHARACTERIZATION UNIT	DATA ANALYSIS UNIT
Bob Coffey, MD	Lead: **Martha Shrubsole**, PhD, Epidemiologist	Lead: **Ken Lau**, PhD, scRNA-seq	Lead: **Qi Liu**, PhD, Bioinformatics
Ken Lau, PhD	Lead: **Kay Washington**, MD PhD, Pathologist	Lead: **Cindy Sears**, MD, Microbiome	Lead: **Yu Shyr**, PhD, Biostatistics
Martha Shrubsole, PhD	Co-I: **Reid Ness**, MD, Gastroenterologist	Co-I: **Gregor Neuert**, PhD, smFISH	Co-I: **Lynne Berry**, PhD, Data Distribution
	Co-I: **Tim Geiger**, MD, Colorectal Surgeon	Co-I: **Bob Coffey**, MD, MxIF	Co-I: **Simon Vandekar**, PhD, Image Analysis
	Co-I: **Tim Su**, MD PhD, Pathologist	Co-I: **Bob Anders**, MD PhD, Immunology	Co-I: **Quanhu Sheng**, PhD, Software Engineering
	Co-I: **Qiuyin Cai**, MD, PhD, Biorepository	**Co-I: Joe Roland**, PhD, Digital Histology	

COLON MAP Participants



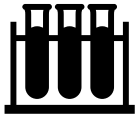
- Recruited from scheduled colonoscopies and surgical resections for large polyps at Vanderbilt

- Integrated systems to facilitate efficient recruitment of participants, collection of biospecimens, and management of data and biospecimens



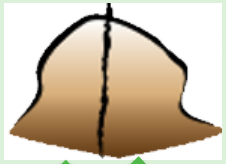
- Stratified sampling design to enhance racial/ethnic diversity (~24%)

- Surveys for individual-level exposure and health history



- Biospecimen collection

COLON MAP Tissue Collection



Fresh

FFPE

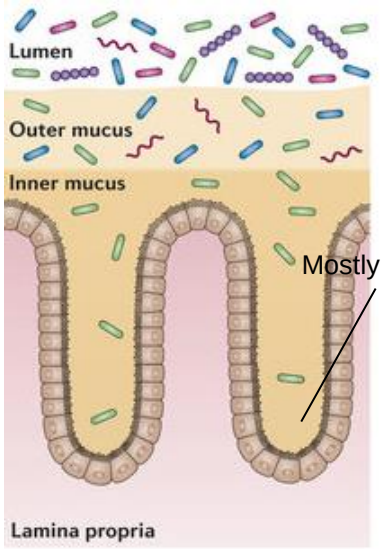
scRNA-seq
Biofilm
Frozen
Colonoid

Diagnosis

MxIF, MxIHC

- Standard of care colonoscopy
- All polyps removed
  - FFPE for clinical diagnosis
  - Other portion reserved for research
- Collection of 8 normal-appearing colon biopsies (4 R, 4 L)
- Standardized research pathology review of all FFPE polyps

Colon architecture



(Donaldson et al. 2015)

Microbes affects surface and crypt cells differently

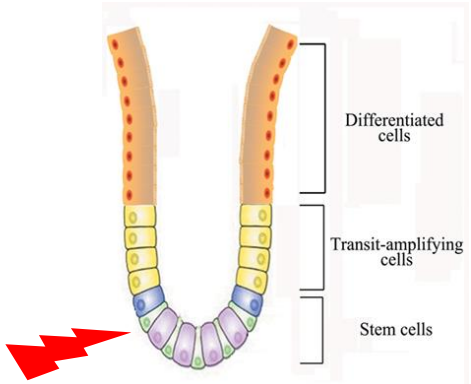
Models of GI tumorigenesis

Stem tumorigenic state

Crypt stem cells as the cells-of-origin of intestinal cancer

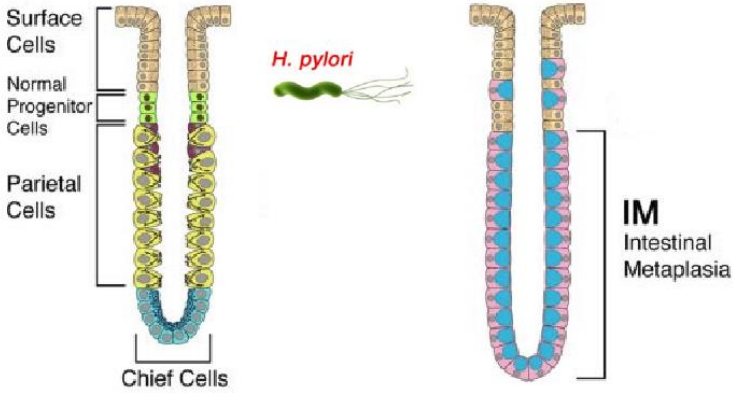
Nick Barker, Rachel A. Ridgway, Johan H. van Es, Marc van de Wetering, Harry Begthel, Maaike van den Born, Esther Danenberg, Alan R. Clarke, Owen J. Sansom & Hans Clevers

Nature **457**, 608–611(2009) | Cite this article



Bi-allelic APC mutation

Non-stem tumorigenic state



(Spasmolytic polypeptide-expressing metaplasia - Goldenring and Nomura, 2006)

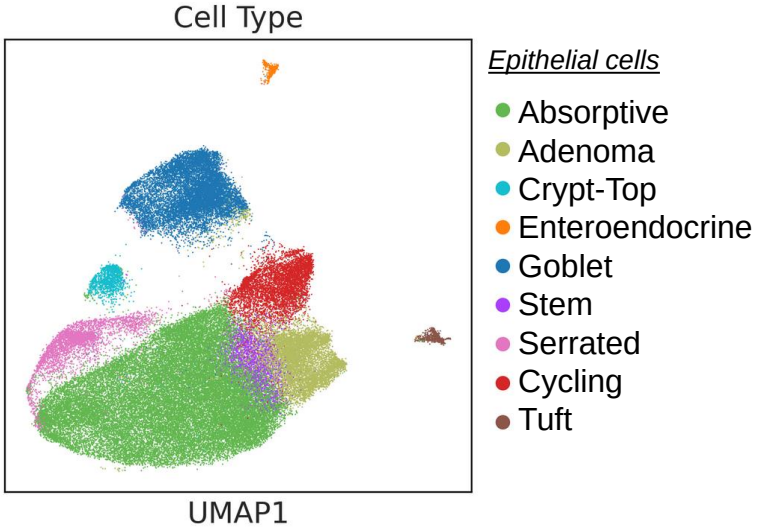
Metaplasia – transition of a differentiated cell into regenerative non-native cell state

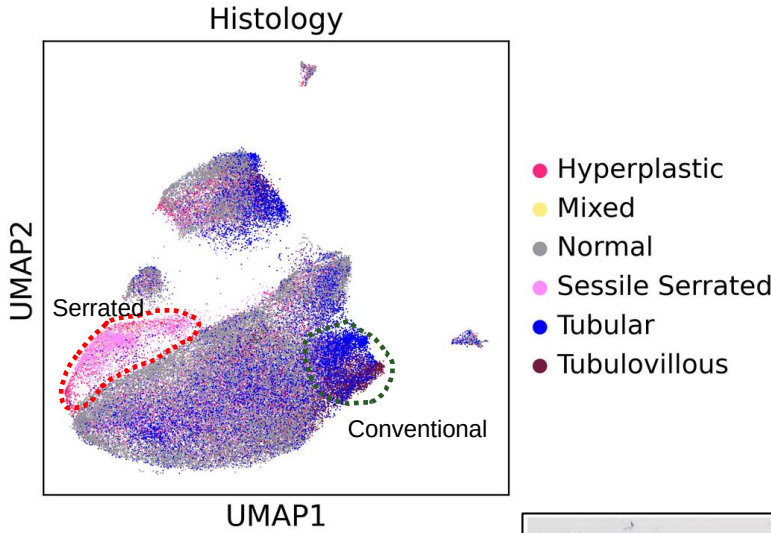
Key Questions

1. Can we find evidence of an alternative origin of tumorigenesis (aside from stem cells) in human colon tumors?

2. If so, do these tumors exhibit a different immune tone and microenvironment?

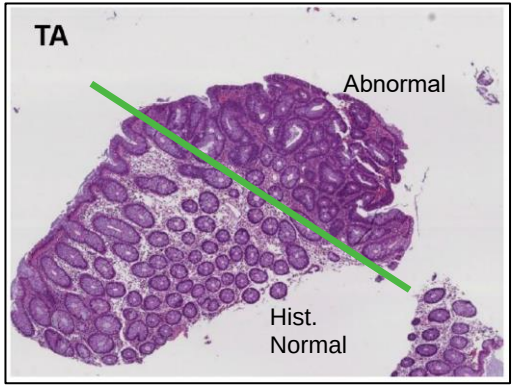
scRNA-seq co-embedding with normal specimens to identify transcriptionally abnormal epithelial cells





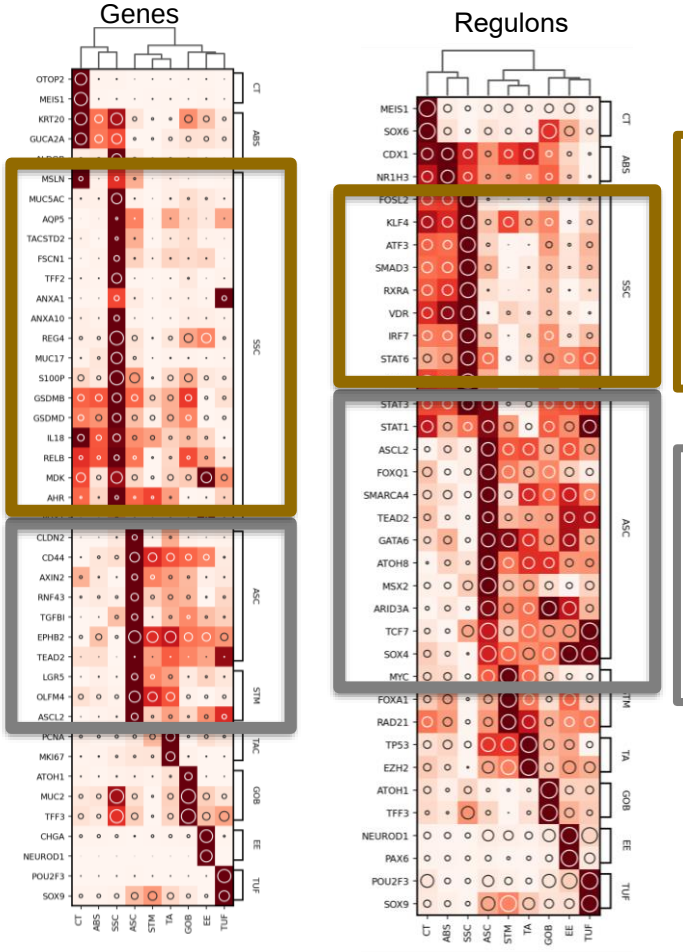
Bob Chen

Training set (30 participants; 69 samples; 75,000 cells)
- 14 adenomas
- 13 serrated
- 3 CRC
- Accompanying normal



Chen B, Scurrah CR, *et al Cell* DOI:https://doi.org/10.1016/j.cell.2021.11.031

Adenoma cells (ASCs) and serrated cells (SSCs) exhibit different pathway profiles



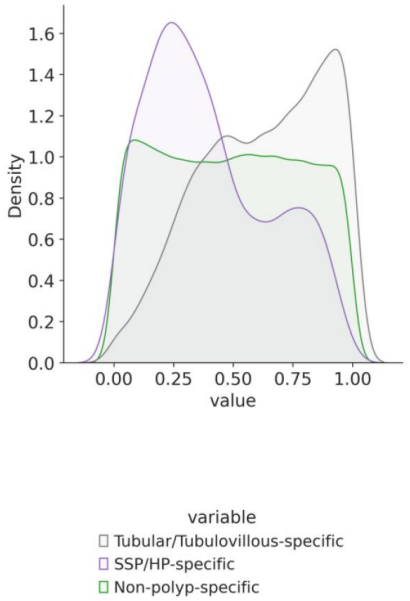
Serrated-specific cells

- Lack WNT-pathway activation
- Differentiated cell characteristics (e.g. KRT20)
- Low inferred stem potential

AD-specific cells

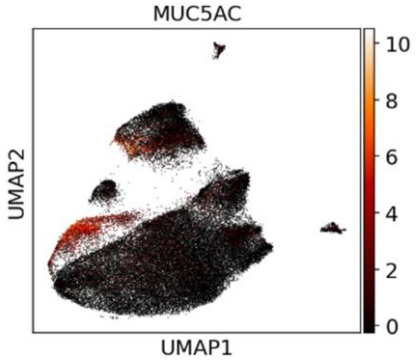
- WNT pathway activation
- Transcriptomic profile similar to normal stem cells
- High predicted stem potential

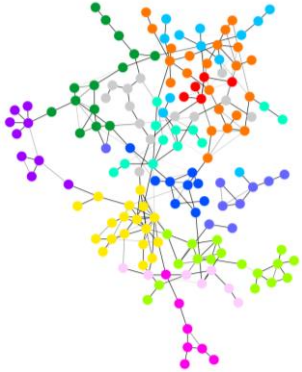
CytoTRACE



Serrated cells activate gene networks related to damage response and metaplasia

Metaplasia

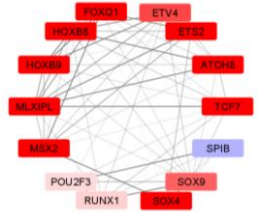


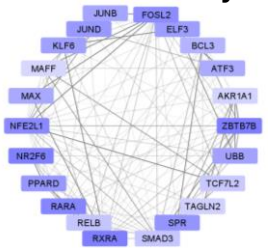


- Transcriptomically resemble absorptive-lineage cells but express selected goblet cell genes (mucins)
- Enriched for non-colonic genes expressed in other endodermal organs

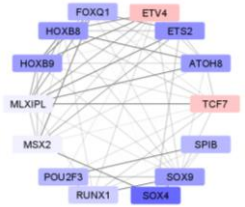
Stem **Inflammatory**

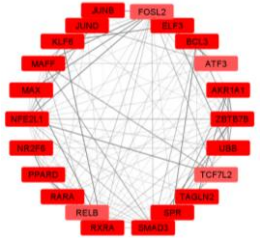
Adenoma





Serrated

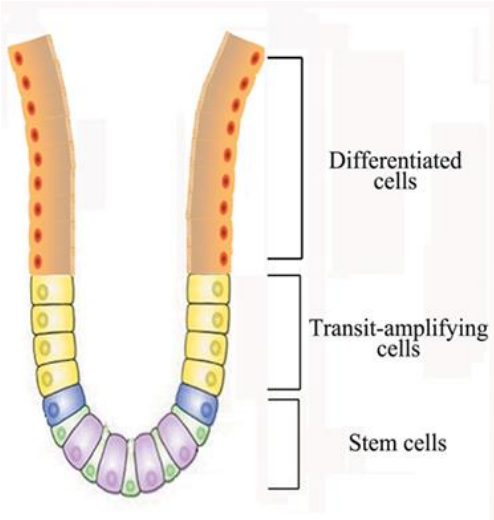




- Loss of caudal regional identity and re-expression of embryogenic genes
- Signature for inflammatory response to epithelial damage

Top-down or Bottom-up Model of Tumorigenesis

Adenomas



Serrated Polyps

Stem cell expansion and maintenance of caudal identity in conventional adenoma

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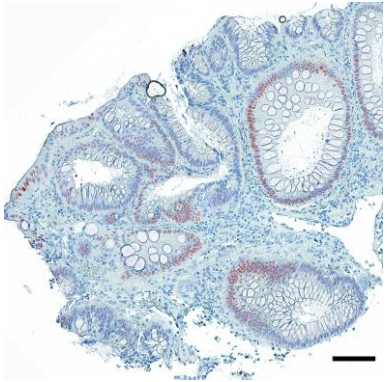
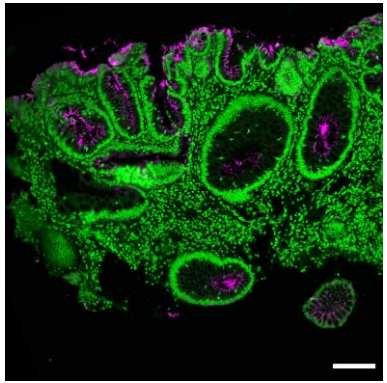
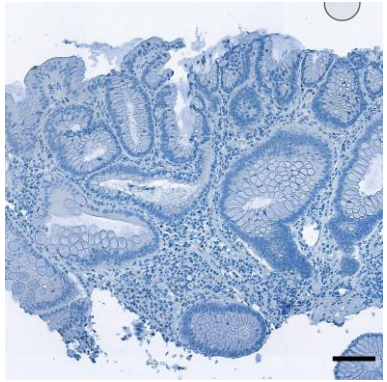
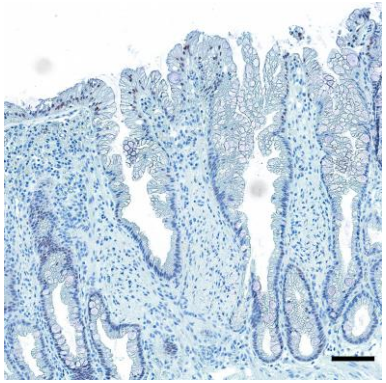
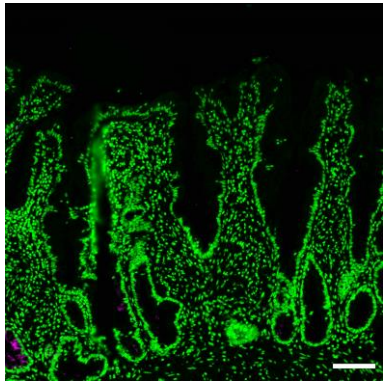
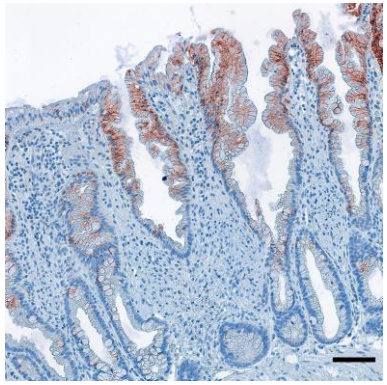
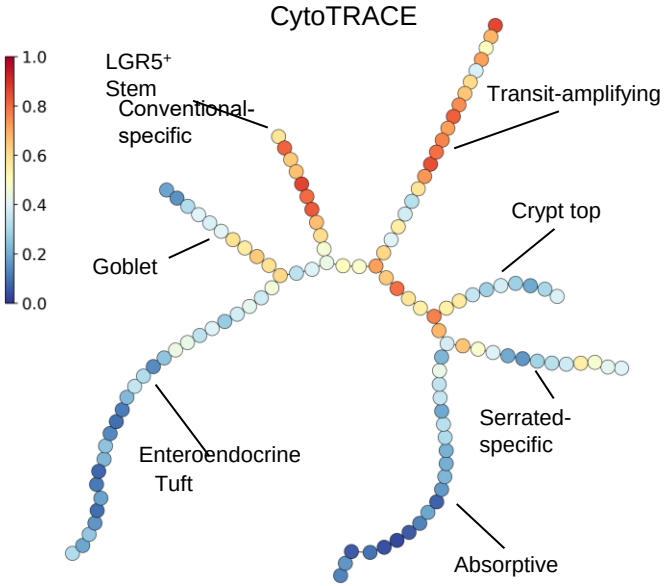
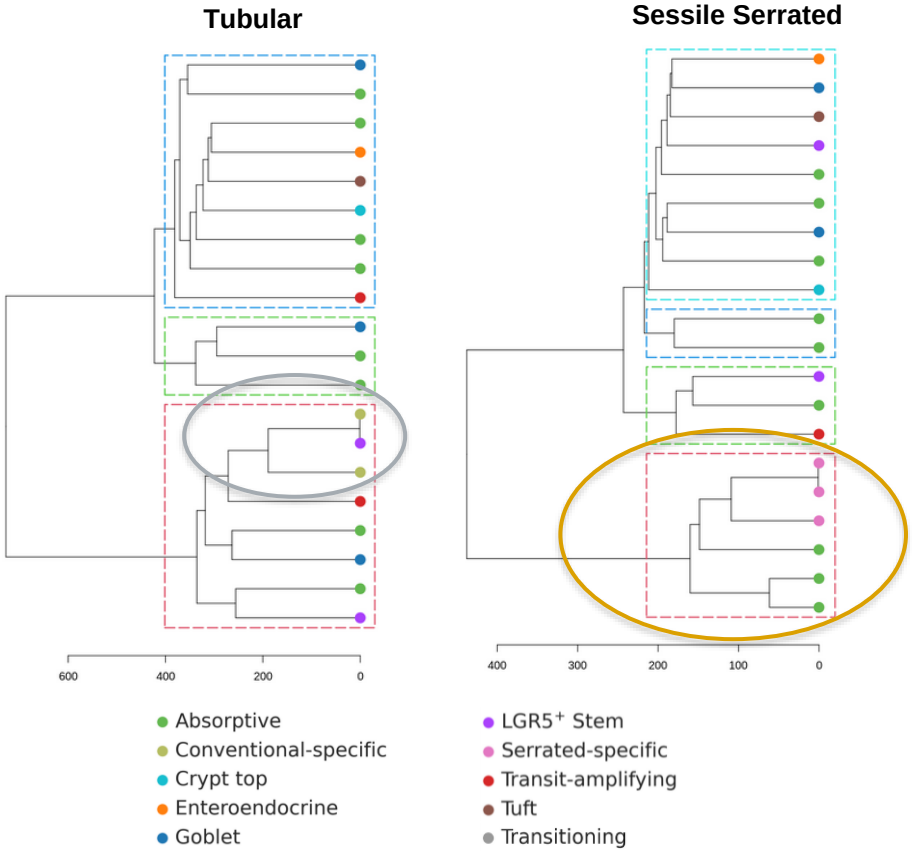
Eliot McKinley

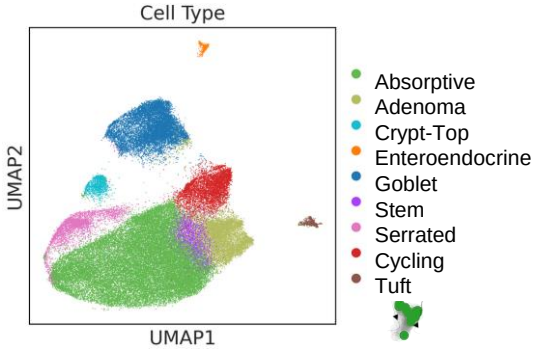
	CDX2 (regional ID)	OLFM4 (Stem)	MUC5AC (metaplasia)
Adenoma			
Sessile Serrated			

Evidence of non-stem origin of serrated cells: shared phylogeny with differentiated cells

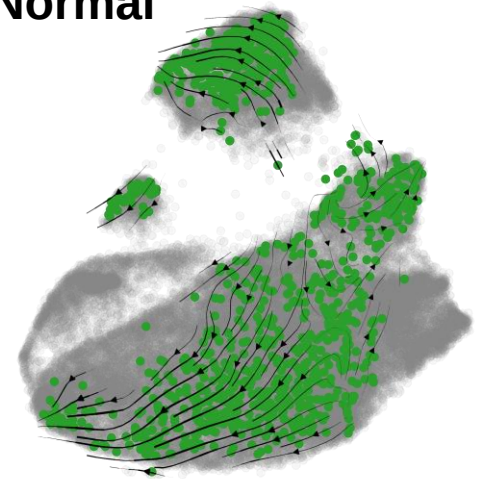




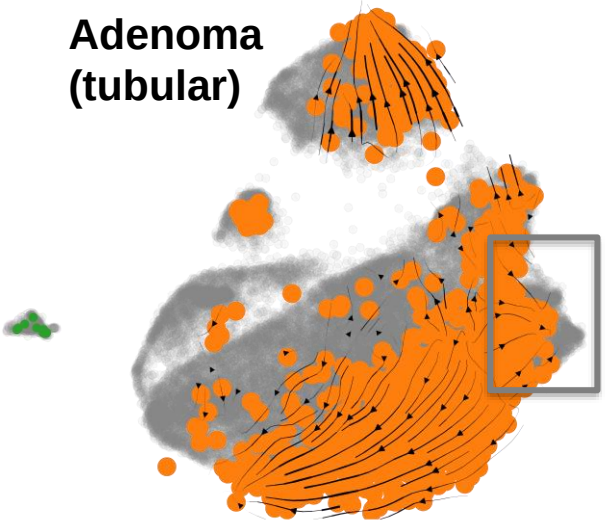
Evidence of non-stem origin of serrated cells: velocity suggests non-stem origin



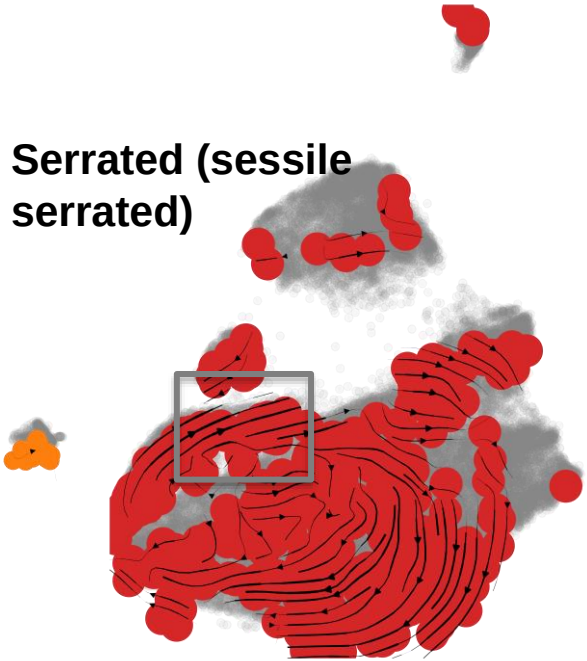
Normal



Adenoma (tubular)

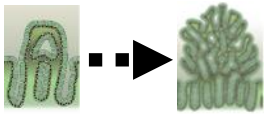


Serrated (sessile serrated)

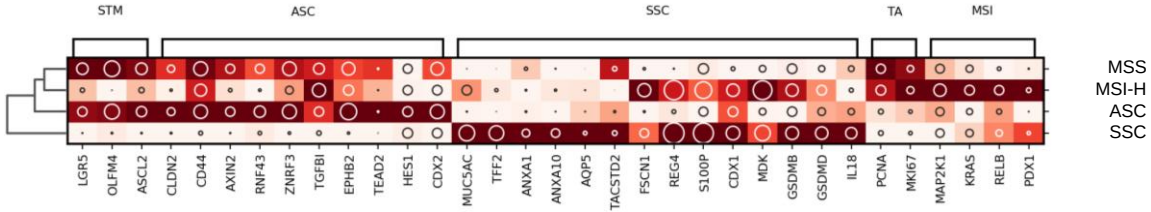


Metaplastic signatures of serrated polyps are carried into MSI-H CRC

Serrated Polyps (HP, SSLs)

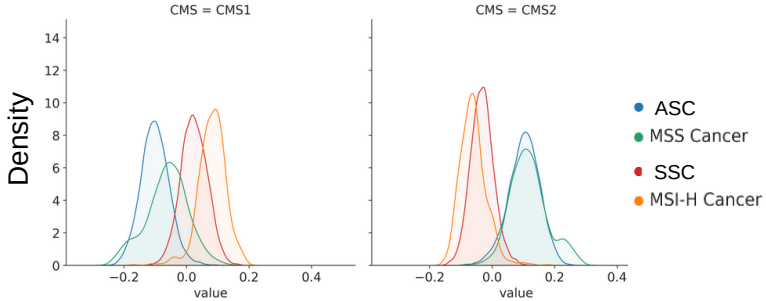


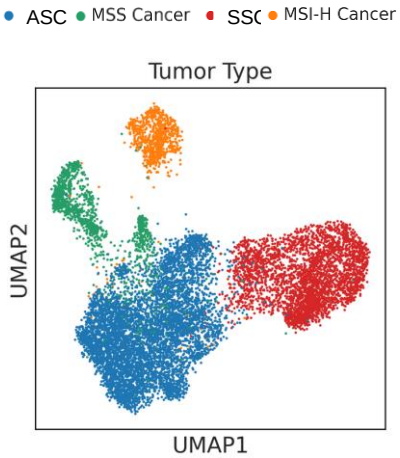
MSI-H Cancer



MSI subtype

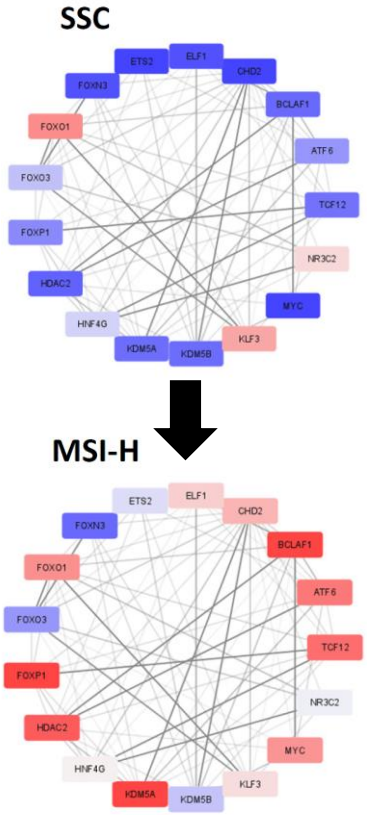
WNT subtype

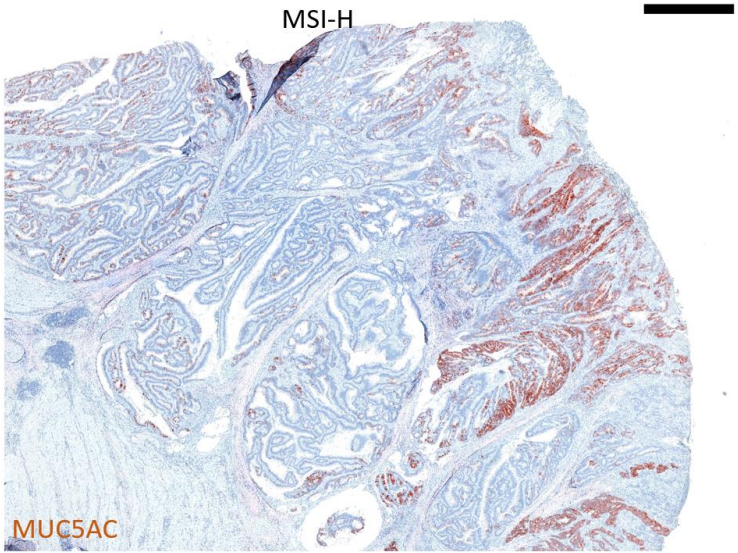


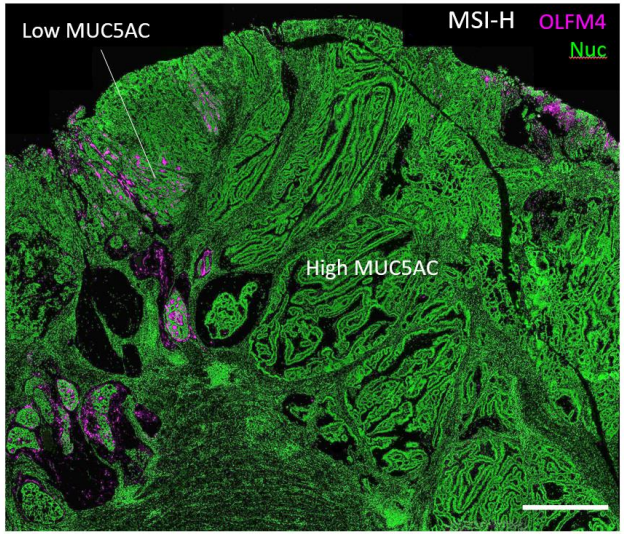


Abnormal epithelial cells

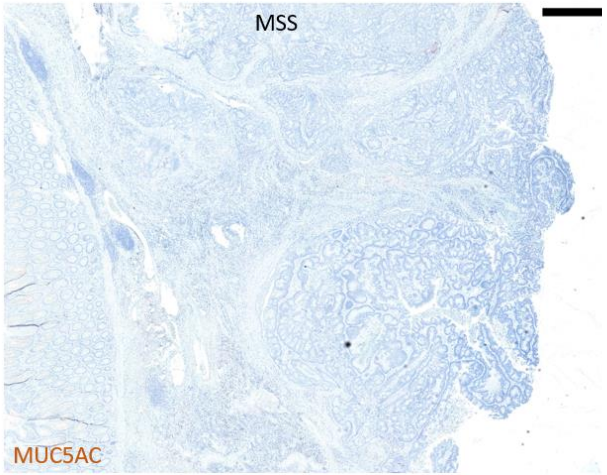
Stem cell program reactivated in MSI-H CRC cells compared with serrated polyp cells

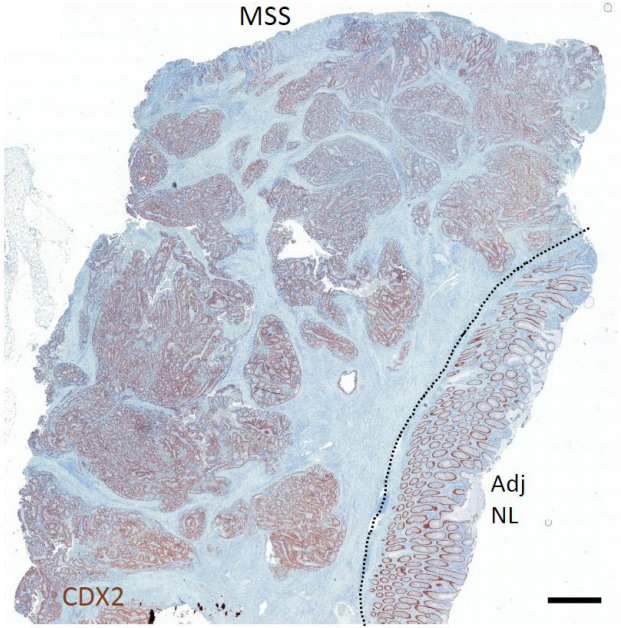




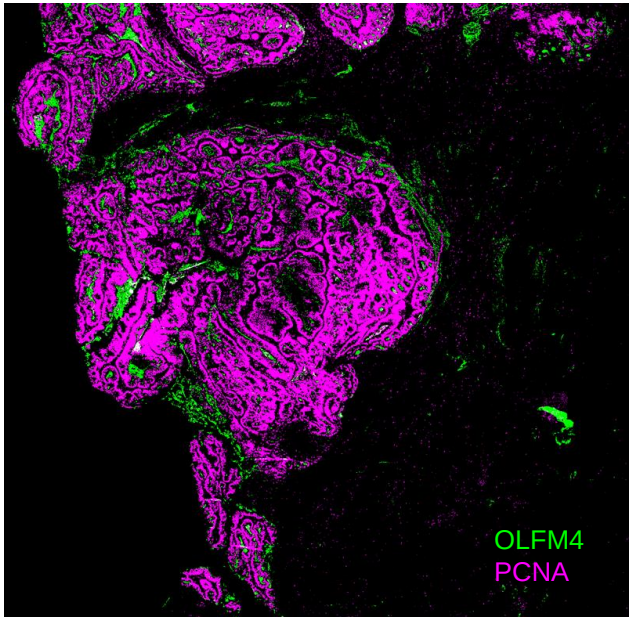


Lack of stem and metaplastic heterogeneity in MSS CRC

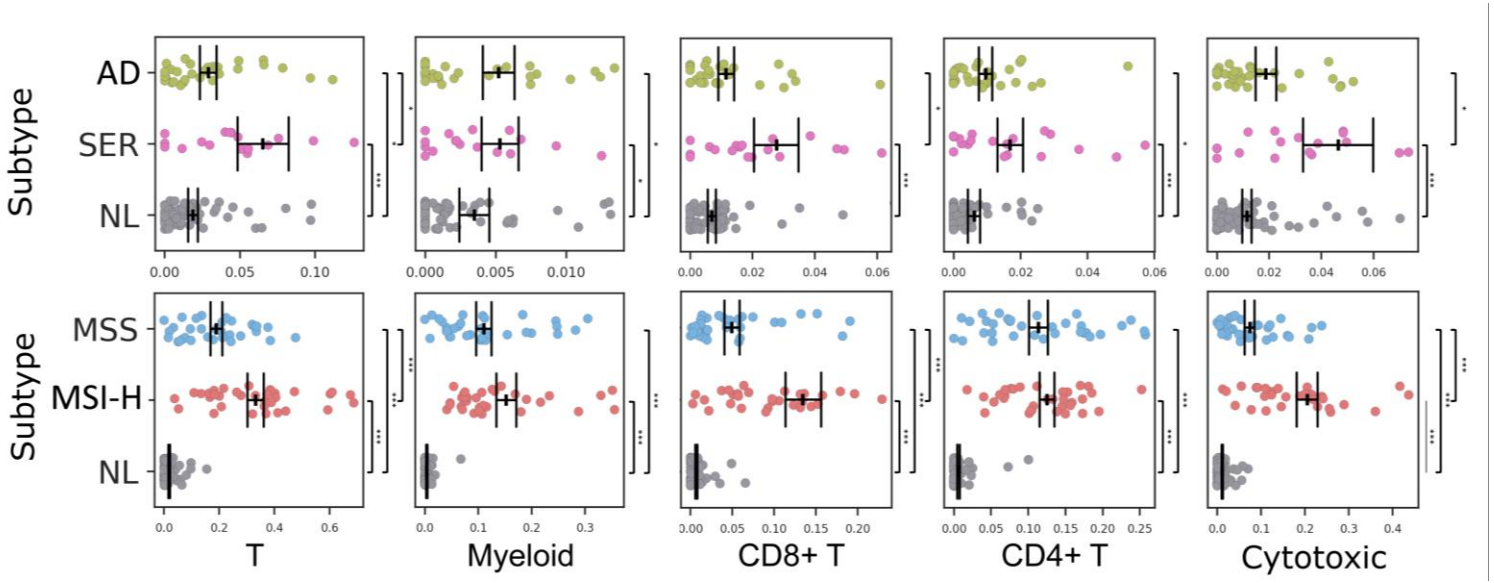




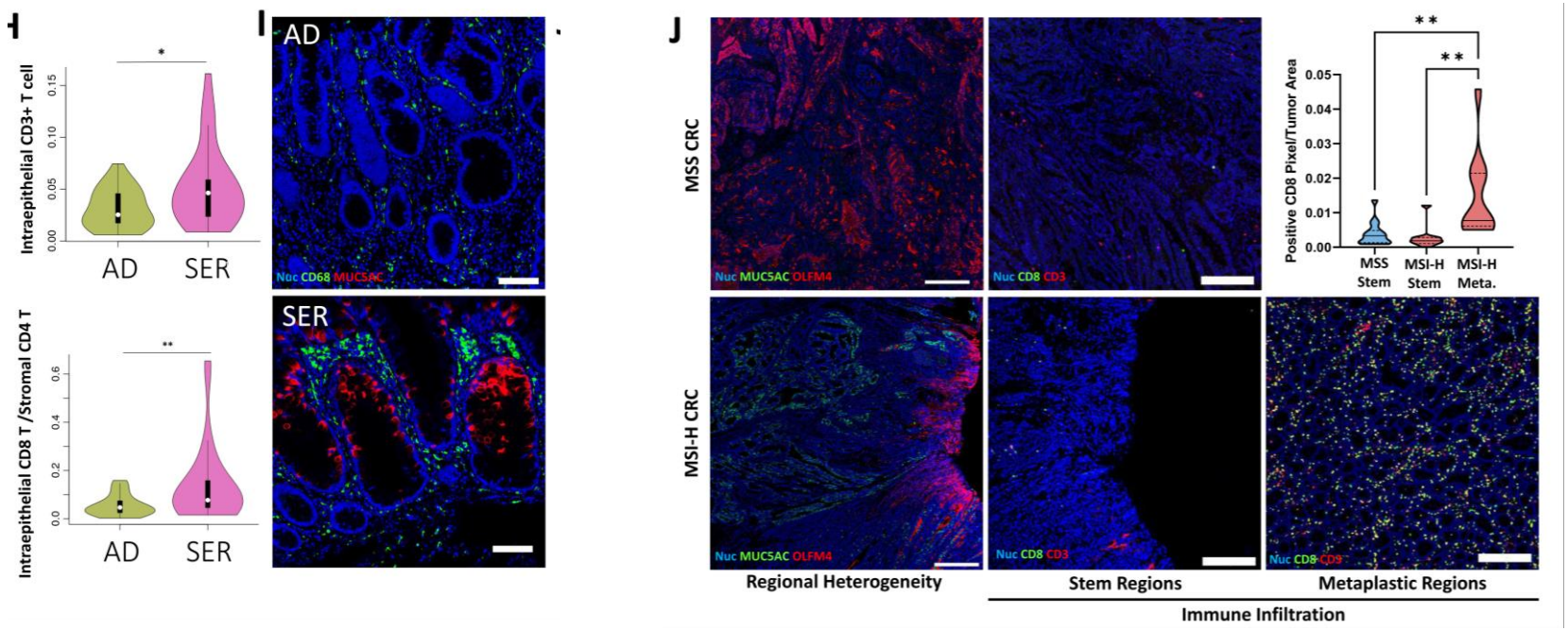
MSS



Cytotoxic immune cells are increased in serrated polyps



Geographical differences in the immune compartment



Functional validation of the tumor cell-differentiation status and the effects on cytotoxic immunity

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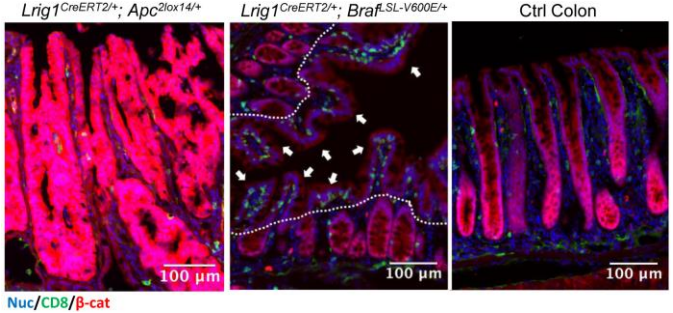
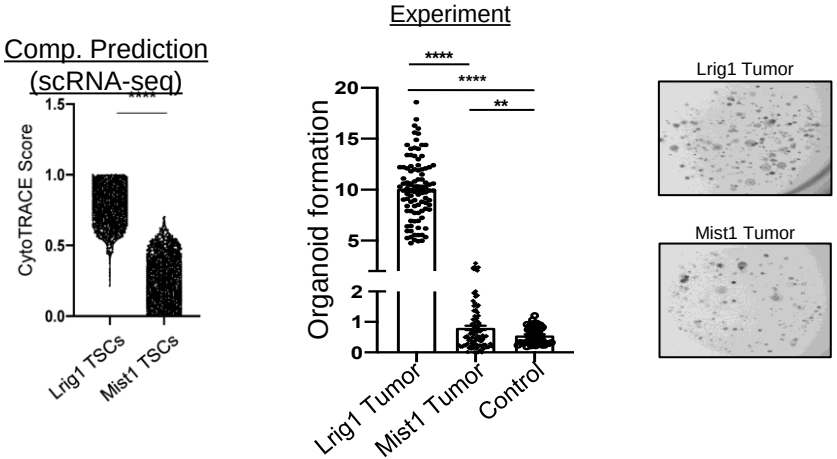
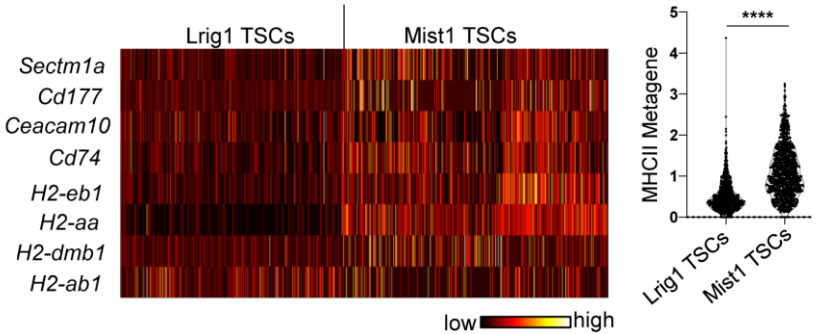
1. Braf mutant villiform lesions show increased CD8+ T cell infiltration

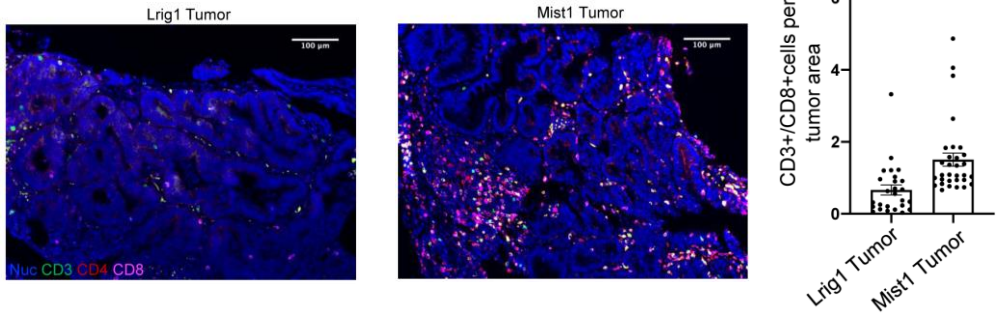


2. Mist1 tumor cells exhibit reduced stemness and cytotoxic immune microenvironment



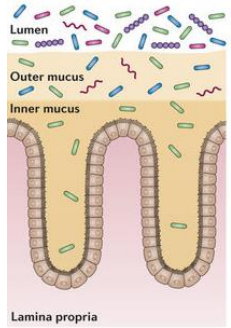
3. Tumor cells exhibit increased expression of antigen presentation machinery inversely proportional to stemness





Summary

- There are tumor-intrinsic differences between conventional and serrated pathways of tumorigenesis
  - Conventional adenomas arise from activation of WNT and associated regenerative programs; serrated polyps arise from mucinous metaplasia in response to damage
  - The origin of conventional adenomas are implicated to be normal stem cells; serrated polyps may develop from committed absorptive cells
  - Serrated polyps present a cytotoxic immune environment preceding hypermutation



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HTAN Trans-Network Projects (TNPs)

Collaborative research projects across HTAN centers and supported by dedicated research funds

Research Advocates

- HTAN Diversity and Inclusion Working Group
- To support contributions of research advocates in HTAN

SARDANA

- Compare imaging methods across centers
- 7 HTAN centers
- Vanderbilt
  - Enlisted CHTN for sample collection
  - scRNA-seq
  - MxIF

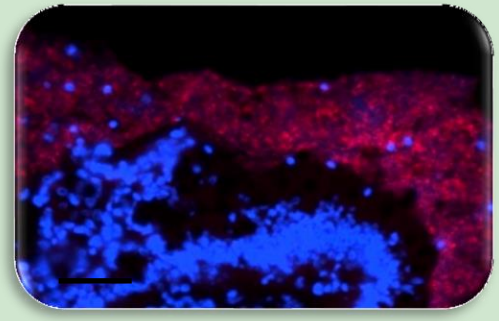
Colorectal Liver Metastasis

- Cross-test platforms and methods and evaluate biological insights into tumor heterogeneity using shared specimens
- 4 HTAN centers

Tissue Cellular Neighborhoods Initiative

- A gold-standard set of cellular neighborhood annotations across tissues
- 5 HTAN centers
- Goals: Open-source and inter-operable tools

Biofilm in CRC TNP



- Initiated new TNP focused on role of biofilms (BF) in CRC
- Collaboration with Stanford and WUSTL
- <u>Malaysia Biofilm Study</u>
  - Led by Dr. Cindy Sears
  - Collection of unique colorectal cancer/tissue samples suitable for biofilm analysis
  - Characterized by FISH for invasive biofilms

- TNP: Serial sections of FFPE tumor
  - FISH (JHU)
  - MxIF (Vanderbilt)
  - Nanostring on regions of interest (Stanford)

Cindy Sears

Julia Drewes

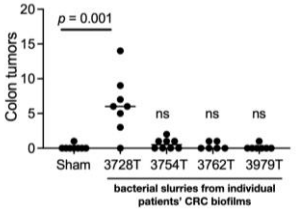
Vanderbilt PCA Contributions to HTAN to Date

- Intentional recruitment plan to optimize diversity
- Extensive characterization of participants and their biospecimens
- Generation of largest single-cell RNA-seq dataset of colorectal polyps
- Deposition of data/publications to DCC and other public resources
- > 15 publications including scientific findings and methods
- Creation of open source tools (e.g dropkick, MIRIAM)
- Leadership of multiple working groups within HTAN
- Initiation of and collaboration in multiple TNPs
- Training and research opportunities for junior investigators
- Leverage of other resources to enhance the scientific goals and impact

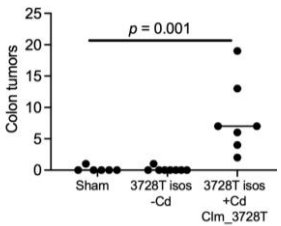
Human colon cancer-derived *C. difficile* strains drive colonic tumorigenesis in germ-free *Apc*$^{Min/+}$ mice

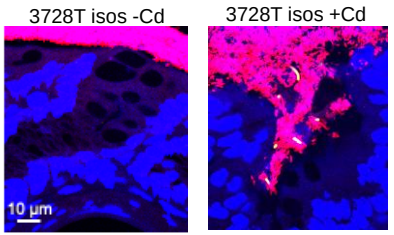
VANDERBILT UNIVERSITY MEDICAL CENTER

1. Variability in tumorigenic capacity of biofilm+ CRC mucosal slurries



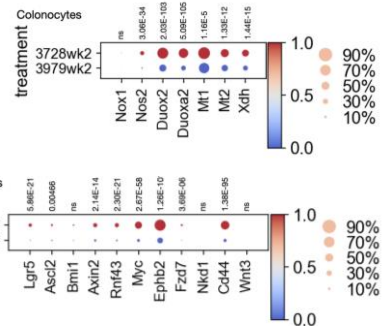
2. *C. difficile* drives tumorigenesis and crypt-invasive biofilm formation of the 3728T isolates (30-bacteria mixture)





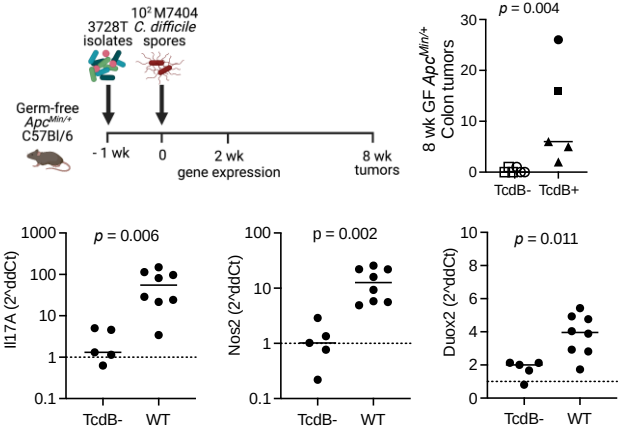
EUB338
C. difficile
DAPI

3. Cell-specific signatures in mice in response to *C. difficile*+ slurry (3728T) vs. *C. difficile*- slurry (3979T) from CRC patients



Cell Type	Analysis	Gene Set	Description
Deep Crypt Secretory	Reactome	R-MMU-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell
Progenitor	Wikipathway	WP222	Cytokines and Inflammatory Response
All epithelial	KEGG	mmu04672	Intestinal immune network for IgA production
Goblet	Wikipathway	WP1253	Type II interferon signaling
Colonic Stem	KEGG	mmu05321	Inflammatory bowel disease
Neutrophil	KEGG	mmu05204	Chemical carcinogenesis

4. Tumorigenesis at 8 wk p.i., and IL-17 and ROS induction at 2 wk p.i. is dependent on *C. difficile* toxin TcdB



Drewes, Chen, Markham… Housseau, Lau, Sears 2022 in press *Cancer Discovery*

Jie Chen

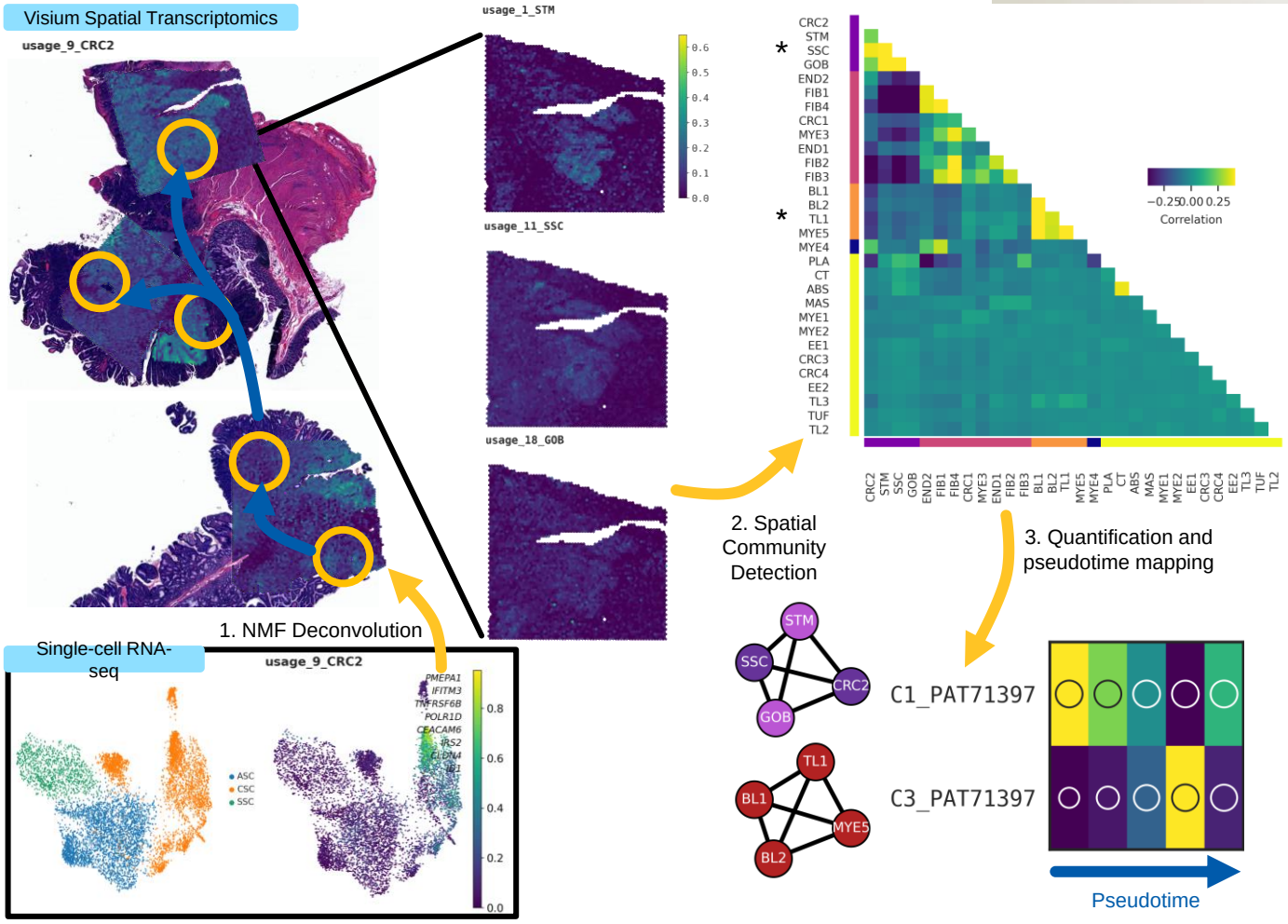
Nick Markham

Julia Drewes

Next Steps and On-going Activity

- Expanded sample size for scRNA-seq
- MxIF, MxIHC for spatial relationships and methods
- Follow-up of participants for future outcomes
- Colonoids
- Newsletter and lay summaries
- Application for additional funding to support hypothesis-testing and validation in animal models
- Spatial transcriptomics

Spatial Transcriptomics



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Tissue Pathology Shared Resource
Digital Histology Shared Resource
Epithelial Biology Center

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Julia Drewes

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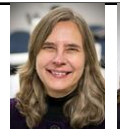
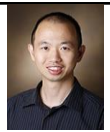
COLON MAP LEADERSHIP	
Overall Bob Coffey (contact PI), Ken Lau, Martha Shrubsole	
Biospecimen Acquisition, Processing & Classification Unit Martha Shrubsole, Kay Washington	 
Molecular, Cellular & Tissue Characterization Unit Ken Lau, Cindy Sears (Hopkins)	 
Data Processing, Analysis & Modeling Unit Qi Liu, Yu Shyr